DATA EVALUATION RECORD

CAPHRA/β-CYFLUTHRIN

Study Type: OCSPP Non-Guideline; *In Vitro* Metabolism Kinetics

EPA Contract No. EP-W-16-018
Task Assignment No.: 32-3-013 (MRID 50600307)

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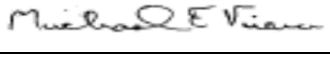
Project Manager: Michael E. Viana, Ph.D., DABT	Signature: Date:	 10/05/2018

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STUDY TYPE: OCSPP Non-guideline; *In Vitro* Metabolism Kinetics.

PC CODE: 118831

DP BARCODE: D448281

TXR#: 0057772

TEST MATERIAL (PURITY): β-Cyfluthrin (99.0% a.i.)

SYNONYMS: Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

CITATION: Brown, S. (2018) β-cyfluthrin: a study to determine the kinetics of metabolism of β-cyfluthrin in rat and human plasma, rat and human liver microsomes and rat and human liver cytosol; Final Report. Concept Life Sciences Dundee, Dundee Technopole, Dundee, United Kingdom. Laboratory Project ID: CXR1722-II β-cyfluthrin, May 31, 2018. MRID 50600307. Unpublished.

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EXECUTIVE SUMMARY: In a non-guideline, *in vitro* metabolism study (MRID 50600307), the apparent intrinsic clearance (CL_{int}) of β-cyfluthrin (99.0% a.i.; Batch # 0507200901) was determined in human liver microsomes, liver cytosol, and plasma, as well as juvenile and adult rat liver microsomes, liver cytosol, and plasma (See Appendix; Source of test systems). Initially, an analytical method development was conducted with β-cyfluthrin and bifenthrin (95.9% a.i.; Batch # PL15-0330) as an internal standard to establish LC-MS/MS chromatography and ionization conditions (identical to the LC-MS/MS chromatography and ionization conditions determined for bifenthrin [MRID 50600301, concurrently reviewed]), as well as the limit of quantitation, linearity, and recovery. Next, preliminary experiments in human and rat microsomes and plasma were conducted with β-cyfluthrin to establish appropriate experimental time points, stability in incubation samples stopped with bifenthrin after refrigerated storage (2-8°C) for eight days, stability of stock solutions after refrigerated storage (2-8°C) for fourteen or 28 days, and stability of QC samples prepared from a β-cyfluthrin stock solution after refrigerated storage (2-8°C) for eleven or 25 days. Finally, the main study was conducted by incubating human and juvenile (15-day and 21-day old) and adult (90-day old) Sprague-Dawley rat liver microsomes, liver cytosol, and plasma in the presence and absence of NADPH with 0.1 μM β-cyfluthrin. Liver microsomes and cytosols were incubated at 0.1 mg/mL; plasma was

incubated at a dilution of 1:1000. The 1:1000 dilutions yielded protein concentrations of 0.046, 0.048, and 0.077 mg/mL for the 15-day, 21-day, and 90-day old rat plasma dilutions, respectively, and 0.082 mg/mL for human plasma. Microsomal metabolism in the presence of NADPH reflected both NADPH-dependent cytochrome P450 (CYP) metabolism and NADPH-independent carboxylesterase (CES) metabolism. In contrast, NADPH-free incubations measured only CES metabolism. CYP-only metabolism was estimated by the difference between the two determined metabolic rates. Because CYP activity is not observed in liver cytosol and plasma, only β-cyfluthrin metabolism by CES enzymes was investigated in these compartments. β-cyfluthrin metabolism, determined as the loss of β-cyfluthrin in the samples, was evaluated by LC-MS/MS. Estimates of CL_{int} in microsomes and cytosol were determined from the decline in substrate concentration over time with the following equation:

$CL_{int} = K_{dep} \times$ incubation volume (mL)/ mg protein,

where K_{dep} is the slope of the regression of the natural logarithm of the percentage substrate remaining against time.

Estimates of CL_{int} in plasma were determined from the decline in substrate concentration over time with the following equation:

$CL_{int} = K_{dep} \times$ incubation volume (mL)/ mL plasma.

RESULTS

Analytical Method Development: Liquid chromatographic separation was conducted with a C18 column (50 × 2 mm) at 50°C. The mobile phase was 85% methanol containing 0.1% formic acid and 15% 10 mM ammonium formate in water (isocratic) with a flow rate of 0.45 mL/min. Mass spectrometry was conducted with positive ion electrospray ionization. The run time was 1.8 min with an injection volume of 1 µL. A calibration line spanning 0.02-0.3 µM β-cyfluthrin was established, with the lower limit of quantification determined at 0.02 µM β-cyfluthrin.

Preliminary Experiments: Duplicate incubations with 0.1 µM and 0.2 µM β-cyfluthrin in human and 90-day old rat liver microsomes and plasma were conducted over a time course of 0, 5, 15, 30, and 60 min. Microsomal incubations were conducted both in the presence and absence of NADPH; plasma incubations were conducted without addition of NADPH. For 90-day old rat microsomes in the presence of NADPH, 80.3% of the 0.1 µM β-cyfluthrin and 78.5-79.8% of the 0.2 µM β-cyfluthrin were metabolized after 15 min; all subsequent time points were below the lower limit of quantification (BLQ). For 90-day old rat microsomes in the absence of NADPH, β-cyfluthrin metabolism was slower, with a maximum of 50.4-55.6%% of the 0.1 µM β-cyfluthrin and 45.8-46.3% of the 0.2 µM β-cyfluthrin metabolized after 60 min. Human microsomes in the presence of NADPH demonstrated slightly slower metabolism of β-cyfluthrin, with a maximum of 73.6-74.0% depletion of the 0.1 µM solution after 60 min and 73.9-75.7% of the 0.2 µM solution metabolized after 60 min. For human microsomes in the absence of NADPH, β-cyfluthrin metabolism was similar to metabolism rat microsomes in the absence of NADPH, with a maximum of 55.7-56.9% of the 0.1 µM β-cyfluthrin and 57.5-58.1% of the 0.2 µM β-cyfluthrin metabolized after 60 min. For rat plasma, 31.3-32.6% of the 0.1 µM β-

cyfluthrin and 31.7% of the 0.2 µM β-cyfluthrin were metabolized after 60 min. Minimal metabolism of β-cyfluthrin was observed in human plasma (1.0-3.8% of the 0.1 µM β-cyfluthrin and 8.1-9.0% of the 0.2 µM β-cyfluthrin were metabolized after 60 min). Incubation samples stored for eight days did not exhibit acceptable stability, so samples were extracted on the same day that incubations were conducted for the main study. Stock solutions stored fourteen days were found to exhibit acceptable stability, but solutions stored for 28 days did not, so solutions were stored for no more than fourteen days. Stability of the QC samples prepared from solutions stored for eleven or 25 days were determined to be acceptable.

Main Study Experiments: β-cyfluthrin was well metabolized by the CYP enzymes of the rat liver microsomes, with activity similar between the 90-day and 21-day old rats and decreased in the 15-day old rats, with human liver microsomes demonstrating no CYP activity (Appendix, Tables 1 and 2). All enzyme activity in human microsomes regarding β-cyfluthrin was attributed to CES enzymes. Estimates of CL_{int} for CYP activity were 2.72 mL/min/mg for 90-day old rat, 2.52 mL/min/mg for 21-day old rat, and 1.06 mL/min/mg for 15-day old rat microsomes. Estimates of CL_{int} for CES activity were 0.21 mL/min/mg for 90-day old rat, 0.23 mL/min/mg for 21-day old rat, 0.08 mL/min/mg for 15-day old rat, and 0.65 mL/min/mg for human microsomes. CL_{int} from rat liver CES enzymes were 7.7%, 9.1%, and 7.5% of CYP enzymes in the 90-day, 21-day, and 15-day old rat liver microsomes, respectively.

Metabolism of β-cyfluthrin was observed in all cytosol (Appendix, Table 3) and plasma samples, except human plasma (Appendix, Table 4). Activity was similar between the 90-day and 21-day old rats and decreased in the 15-day old rats for cytosol, and activity decreased with decreasing age of the rats for plasma. The estimate of CL_{int} in human cytosol was greater than that in the 90-day and 21-day old rats by a factor of approximately two. Estimates of CL_{int} in cytosol were 0.19 mL/min/mg for 90-day old rat, 0.17 mL/min/mg for 21-day old rat, 0.07 mL/min/mg for 15-day old rat, and 0.44 mL/min/mg for human cytosol. Estimates of CL_{int} in plasma were 11.3 mL/min/mL for 90-day old rat, 2.22 mL/min/mL for 21-day old rat, and 0.64 mL/min/mL for 15-day old rat.

COMMENTS: β-cyfluthrin metabolism was attributed to both CYP and CES enzymes present in rat liver microsomes (primarily CYP) but was attributed only to CES enzymes in human liver microsomes (at least three-fold greater compared to activity in rat liver microsomes). The rates of metabolism in rat plasma were greater in the 90-day old rats compared to the ≤21-day old rats, and metabolism in rat liver microsomes and cytosol was greater in ≥21-day old rats compared to 15-day old rats. β-cyfluthrin metabolism in human liver cytosol was at least two-fold greater compared to activity in rat liver cytosol, but no metabolism was observed in human plasma.

APPENDIX:

Source of test systems:

Rat: Liver tissue and plasma from 15-day, 21-day, and 90-day old Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were shipped to the performing laboratory. Pooled liver microsomal and cytosolic fractions were prepared for each age group (Study Number CXR1572).

Human: Human liver microsomes (Lot # 38290) and cytosol (Lot # 38290) were purchased from Corning B.V. Life Sciences, Amsterdam, The Netherlands. Human plasma (Lot # BRH1194688) was purchased from Sera Laboratories International, Haywards Heath, West Sussex, UK.

TABLE 1 β-cyfluthrin Microsomes Summary Data Main Study Experiments

Species	Experiment Date	Concentration β-cyfluthrin µM	[1]NADPH	K_{dep} min^{-1}	$t_{1/2}$ min	CL_{int} mL/min/mg
Rat 90 day	20-Oct-17	0.1	+	0.345	2.01	3.45
			-	0.0208	33.3	0.208
			CYP	0.325	2.14	3.25
Rat 90 day	02-Nov-17	0.1	+	0.241	2.88	2.41
			-	0.0208	33.3	0.208
			CYP	0.220	3.15	2.20
Rat 21 day	20-Oct-17	0.1	+	0.321	2.16	3.21
			-	0.0295	23.5	0.295
			CYP	0.292	2.37	2.92
Rat 21 day	02-Nov-17	0.1	+	0.228	3.04	2.28
			-	0.0161	43.1	0.161
			CYP	0.212	3.27	2.12
Rat 15 day	24-Oct-17	0.1	+	0.0964	7.19	0.964
			-	0.00737	94.1	0.0737
			CYP	0.0890	7.79	0.890
Rat 15 day	02-Nov-17	0.1	+	0.132	5.27	1.32
			-	0.00865	80.1	0.0865
			CYP	0.123	5.64	1.23
Human	24-Oct-17	0.1	+	0.0710	9.76	0.710
			-	0.0720	9.63	0.720
			CYP	[2]Not calculated		
Human	02-Nov-17	0.1	+	0.0619	11.2	0.619
			-	0.0574	12.1	0.574
			CYP	[2]Not calculated		

[1] Incubations + NADPH were conducted to determine metabolism due to CYP + CES enzymes. Incubations -NADPH were conducted to determine CES metabolism. CYP activity was estimated as the difference between total enzymatic activity (CYP+CES) and CES only activity

[2] Rates of metabolism were similar for incubations + and – NADPH. It is therefore assumed that metabolism is due to CES enzymes and hence no rate for metabolism by CYP enzymes could be determined.

(copied from page 23 of MRID 50600307)

TABLE 2 β-cyfluthrin Microsomes Summary Data Main Study Experiments

Species	Concentration β-cyfluthrin µM	[1]NADPH	Mean CL_{int} mL/min/mg
Rat 90 day	0.1	+	2.93
		-	0.208
		CYP	2.72
Rat 21 day	0.1	+	2.75
		-	0.228
		CYP	2.52
Rat 15 day	0.1	+	1.14
		-	0.0801
		CYP	1.06
Human	0.1	+	0.664
		-	0.647
		CYP	[2]Not calculated

[1] Incubations + NADPH were conducted to determine metabolism due to CYP + CES enzymes. Incubations -NADPH were conducted to determine CES metabolism. CYP activity was estimated as the difference between total enzymatic activity (CYP+CES) and CES only activity

[2] Rates of metabolism were similar for incubations + and – NADPH. It is therefore assumed that metabolism is due to CES enzymes and hence no rate for metabolism by CYP enzymes could be determined.

(copied from page 24 of MRID 50600307)

TABLE 3 β-cyfluthrin Cytosol Summary Data Main Study Experiments

Species	Experiment Date	Concentration β-cyfluthrin µM	K_{dep} min^{-1}	$t_{1/2}$ min	CL_{int} mL/min/mg	Mean CL_{int} mL/min/mg
Rat 90 day	25-Oct-17	0.1	0.0220	31.5	0.220	0.188
Rat 90 day	03-Nov-17	0.1	0.0155	44.7	0.155	
Rat 21 day	25-Oct-17	0.1	0.0126	55.1	0.126	0.169
Rat 21 day	03-Nov-17	0.1	0.0213	32.5	0.213	
Rat 15 day	25-Oct-17	0.1	0.00667	104	0.0667	0.0675
Rat 15 day	03-Nov-17	0.1	0.00683	102	0.0683	
Human	03-Nov-17	0.1	0.0482	14.4	0.482	0.438
Human	03-Nov-17	0.1	0.0394	17.6	0.394	

TABLE 4 β-cyfluthrin Plasma Summary Data Main Study Experiments

Species	Experiment Date	Concentration β-cyfluthrin µM	K_{dep} min^{-1}	$t_{1/2}$ min	CL_{int} mL/min/mL	Mean CL_{int} mL/min/mL
Rat 90 day	26-Oct-17	0.1	0.0122	56.8	12.2	11.3
Rat 90 day	03-Nov-17	0.1	0.0104	66.5	10.4	
Rat 21 day	26-Oct-17	0.1	0.00267	259	2.7	2.22
Rat 21 day	03-Nov-17	0.1	0.00177	392	1.77	
Rat 15 day	26-Oct-17	0.1	0.00128	542	1.28	0.640
Rat 15 day	03-Nov-17	0.1	None detected			
Human	26-Oct-17	0.1	None detected			None detected
Human	03-Nov-17	0.1	None detected			

(copied from page 25 of MRID 50600307)